Inversion of Stereoselectivity by Applying Mutants of the Hydroxynitrile Lyase from *Manihot esculenta***

Holger Bühler, Burkhard Miehlich, and Franz Effenberger*[a]

The influence of Trp128-substituted mutants of the hydroxynitrile lyase from Manihot esculenta *(MeHNL) on the stereoselectivity of MeHNL-catalyzed HCN additions to aldehydes with stereogenic centers, which yield the corresponding cyanohydrins, is described. In* rac*-2-phenylpropionaldehyde (*rac*-**1**) reactions, wild-type (wtMeHNL) and all MeHNL Trp128 mutants are highly (*S*)-selective toward the (*R*) enantiomer of* rac*-**1**; this results exclusively in (2*S*,3*R*)-cyanohydrin ((2*S*,3*R*)-**2**) with ≥96% de. The (*S*) enantiomer of* rac*-**1**, however, only reacts (*S*)-selectively with wtMeHNL to give (2*S*,3*S*)-**2** with 80% de, whereas with Trp128 mutants, (*R*) selectivity increases with decreasing size of the amino acids exchanged. The MeHNL W128A mutant is exclusively (*R*)-selective, resulting in (2*R*,3*S*)-**2** with 86% de. The reaction behavior of* rac*-phenylbutyraldehyde (*rac*-**5**) is comparable with* rac*-**1**, which also inverts the stereoselectivity from (S) to (R) when the enzyme is exchanged from wtMeHNL to the W128A mutant. Stereogenic centers not adjacent to the aldehyde group, as in **7** and **9**, do not influence the stereoselectivity of MeHNL catalysis, and (S) selectivity is observed in all cases. Stereoselectivity and inversion of stereoselectivity of MeHNL Trp128 mutant-catalyzed cyanohydrin formation can be explained and rationalized with crystal-structure-based molecular modeling.*

Introduction

In the wild-type hydroxynitrile lyase of *Manihot esculenta* (wtMeHNL), a tryptophan residue (Trp128) is situated at the channel entrance to the active site of the enzyme.[1,2] Substitution of this residue by amino acids of decreasing size significantly enlarges the channel entrance to facilitate the access of substrates to the active site and thereby causes a considerable change of substrate specificity.[3]

The enantioselective preparation of (*S*)- and (*R*)-cyanohydrins by using hydroxynitrile lyases (HNLs) as biocatalysts has become a very important and attractive tool in stereoselective organic synthesis.[4] Only very little is known, however, concerning the influence of the substrate stereocenters on the reaction with respect to stereoselectivity of the HNL-catalyzed HCN addition.

Because of important follow-up reactions, for example in Kiliani–Fischer carbohydrate synthesis, the stereoselectivity of HNL-catalyzed cyanohydrin formation of α-oxygenated aldehydes is especially of interest. As could be demonstrated, *O*-allyl-protected α-hydroxyaldehydes are excellent substrates for (*R*)-PaHNL-catalyzed HCN addition and yield the corresponding (*R*)-cyanohydrins with high enantioselectivity (≥94% *ee*),[5a] whereas the unprotected α-hydroxyaldehydes react very unspecifically.[5b]

Riva et al.[6] have intensively investigated the (*R*)-PaHNL-catalyzed cyanohydrin formation of α- and β-substituted aldehydes with respect to stereoselectivity. The authors have also investigated α-alkyl- and α-alkoxy-substituted aldehydes as substrates in the *Hevea brasiliensis* (HbHNL)-catalyzed addition of HCN, which gives the corresponding (*S*)-cyanohydrins.[7] Both enzymes, PaHNL and HbHNL, make no chiral discrimination between the enantiomers of racemic substrates, therefore a kinetic resolution of the starting racemic aldehydes was not possible.[7] The diastereoselectivity of reactions of aldehydes with α-alkyl substituents catalyzed by (*S*)-HbHNL[7b] and (*S*)-MeHNL[2] are relatively high and comparable. With (*R*)-PaHNL as catalyst, HCN addition is totally unselective when racemic α-methyl-substituted aldehydes are used, but highly diastereoselective with α-ethyl substituents.[6b,7b] It is interesting to note that the (*R*)-PaHNL-catalyzed HCN addition to *rac*-2-phenylpropionaldehyde is highly (*R*)-selective with the (*S*) enantiomers (diastereomeric ratio 17.3) but unspecific with the (*R*) enantiomer (diastereomeric ratio 1.6).[6a]

The strong influence of mutations on rates and substrate specificity is very well known from our investigations on (*S*)-MeHNL.[3] We were therefore interested to investigate if and to what extend Trp128-substituted channel mutants of wtMeHNL change the stereoselectivity of HCN addition to α- and β-substituted aldehydes that contain stereocenters.

Results and Discussion

MeHNL-catalyzed HCN addition to *rac*-2-phenylpropionaldehyde

The chemical addition of in situ-prepared HCN to racemic 2-phenylpropionaldehyde (*rac*-**1**) in acetic acid yields racemic

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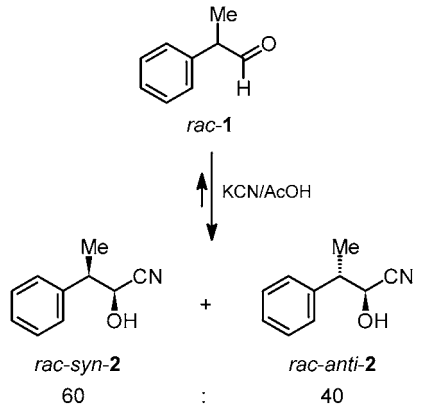
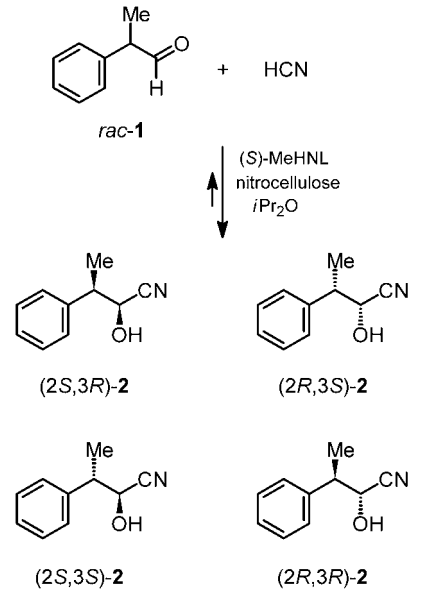
syn- and *anti*-addition products in a ratio of 60:40 (Scheme 1). This result can be explained by the Felkin–Anh model.[8]



Scheme 1. *Chemical HCN addition to racemic 2-phenylpropionaldehyde* rac-***1****. HCN was generated in situ from KCN by acetic acid.*

By using gas chromatogryphy (GC), correlation between a chiral phase (4 peaks, 20:30:20:30) and an achiral phase (2 peaks, 40:60), the relative structure assignment is possible.

In the MeHNL-catalyzed addition of HCN to *rac*-**1,** four diastereoisomers can be formed in principle (Scheme 2).



Scheme 2. *MeHNL-catalyzed HCN addition to* rac-***1****.*

Under standard conditions,[9] with the enzyme adsorbed on nitrocellulose as support and diisopropyl ether as solvent, the reaction is quantitative with all MeHNL mutants after 3 h at room temperature. The following Trp128 MeHNL mutants[3] with decreasing size of amino acids have been applied as biocatalysts in the described cyanohydrin formation:

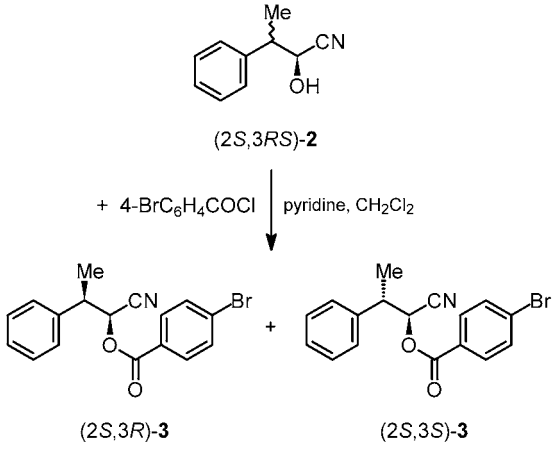
wt > W128Y > W128L > W128C > W128A

The wild-type enzyme (wtMeHNL) is highly (*S*)-selective and preferentially yields the (2*S*,3*R*) and (2*S*,3*S*) diastereomers (Table 1). By changing from the wild-type enzyme to Trp128-substituted mutants, the stereoselectivity changed dramatically with the size of the amino acids introduced. The formation of *syn*-addition products (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** are favored with decreasing size of the amino acids. The almost exclusive (*S*) selectivity of the wild-type MeHNL, is gradually reduced by substitution of Trp128 at the channel entrance by less bulky amino acids (Table 1).

Table 1. *Stereoselectivity of HCN addition to racemic 2-phenylpropionaldehyde* rac-***1*** *catalyzed by wtMeHNL and the MeHNL W128 mutant.*

MeHNL Mutants	Diastereoisomeric composition [%] of **2** (2*R*,3*R*)	(2*R*,3*S*)	(2*S*,3*S*)	(2*S*,3*R*)
wt	0.1	5.2	44.4	50.3
W128Y	0.2	24.4	25.4	50.2
W128L	0.3	37.5	12.2	50.0
W128C	0.5	43.4	6.1	50.0
W128A	1.0	46.2	3.4	49.4

The absolute configuration of the two diastereoisomers obtained in the wtMeHNL-catalyzed reaction of *rac*-**1** with HCN, was elucidated by X-ray crystal-structure determination of the cyanohydrin *O*-(*p*-bromobenzyl) derivatives (2*S*,3*S*)-**3** and (2*S*,3*R*)-**3**, which can be separated by chromatography (Scheme 3, Figure 1).[10]



Scheme 3. *Derivatization of diastereomers (2S,3R)-****2*** *and (2S,3S)-****2****.*

The exclusive formation of the *syn*-addition products (2*S*,3*R*)-**2** and (2*R*,3*S*)-**2** in the reaction of *rac*-**1** catalyzed by the W128A mutant has been confirmed as follows. The ^{1}H and ^{13}C NMR spectra indicate only one reaction product. From GC correlation on a chiral and an achiral phase, and an optical rotation value of 0° (see Experimental Section), the formation of the *syn*-racemate can be deduced. To confirm the *syn*-addition of the MeHNL W128A-catalyzed reaction of *rac*-**1** further, the crude reaction product was hydrolyzed to give the corresponding hydroxy acids *syn*-**4** (Scheme 4).

The racemate *syn*-**4** crystallizes nicely from chloroform to give isolable crystals for X-ray structure determination; this confirms the *syn* configuration (Figure 2).[10]

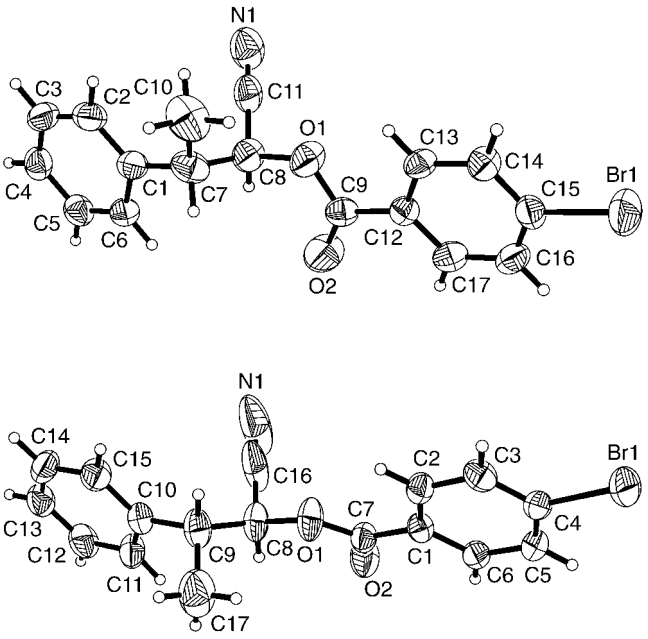
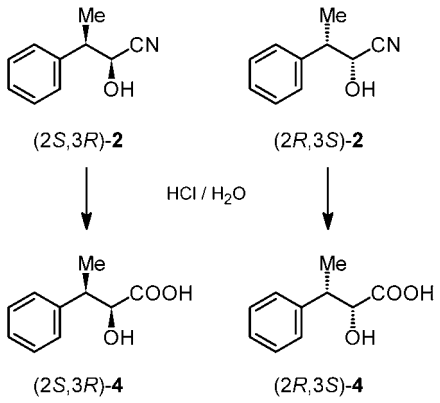


Figure 1. *X-ray structures of the diastereomeric 2-phenylpropionaldehyde cyanohydrin O-(*p*-bromobenzoyl) derivatives **3**: Top: (2S,3S) configuration, bottom: (2S,3R) configuration.*



Scheme 4. *Hydrolysis of* rac-syn-*cyanohydrins **2** to the corresponding* rac-syn-*hydroxy acids **4**.*

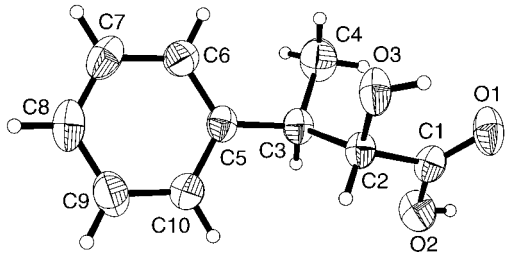


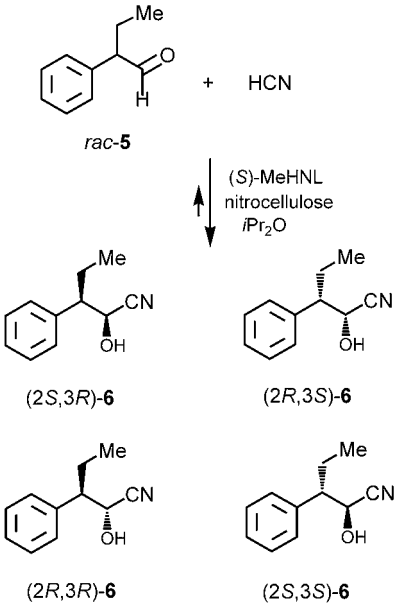
Figure 2. *X-ray structure of* rac-syn-*2-hydroxy-3-phenylbutyric acid (*rac-syn-**4**).*

As could be expected, substitution of Trp128, which covers the channel entrance to the active site of the wild-type MeHNL, by amino acids of reduced size, for example, alanine, improves the rates of conversion of sterically demanding substrates considerably.[3] The inversion of stereoselectivity by mutations in the periphery of the enzyme, as found for the reactions of the (*S*) enantiomer of *rac*-**1** with HCN (Table 1), was unexpected.

MeHNL-catalyzed HCN addition to *rac*-2-phenylbutyraldehyde (*rac*-5)

Similarly to *rac*-**1**, the chemical addition of HCN to racemic 2-phenylbutyraldehyde (*rac*-**5**) preferentially gives the *syn*-addition product (*syn*/*anti* = 66:34). Again, the MeHNL-catalyzed additions of HCN to *rac*-**5** were performed under standard conditions.[9] The sterically more demanding aldehyde **5** (compared to **1**) requires longer reaction times. After 4 h, the reaction of *rac*-**5** with the MeHNL mutants W128Y, W128L, W128C, and W128A is nearly quantitative (Scheme 5, Table 2). wtMeHNL and mutant W128V give cyanohydrin **6** in 87% and 92% yield, respectively, after 4 h.

Whereas (*S*) selectivity was observed in the reaction catalyzed by the wild-type enzyme and the MeHNL W128Y mutant, the stereoselectivity changed markedly for mutants with small-



Scheme 5. *MeHNL catalyzed HCN addition to* rac-*2-phenylbutyraldehyde (*rac-**5**).*

Table 2. *Stereoselectivity of HCN addition to racemic 2-phenylbutyraldehyde* rac-**5** *catalyzed by wtMeHNL and the MeHNL W128 mutants, which give the diastereomeric cyanohydrins **6**.*

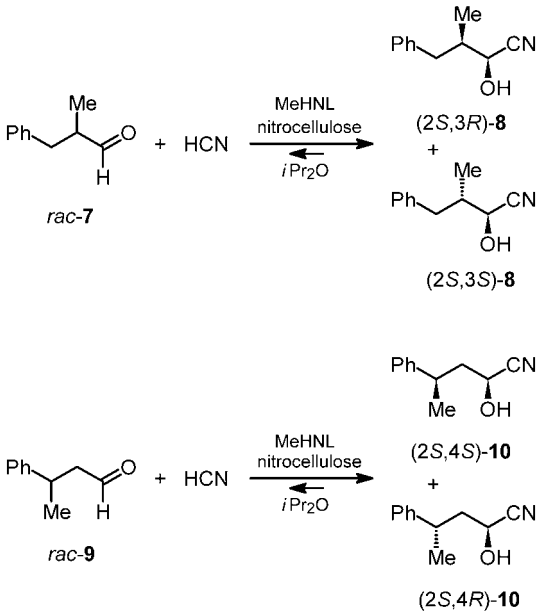
MeHNL Mutants	Diastereoisomeric composition [%] of **6** (2*R*,3*R*)	(2*R*,3*S*)	(2*S*,3*S*)	(2*S*,3*R*)
wt	0.5	4.5	45.8	49.2
W128Y	0.7	4.6	45.6	49.1
W128L	0.8	24.3	25.1	49.8
W128C	0.4	23.4	26.1	50.1
W128A	0.6	34.1	15.0	50.3
W128V	0.0	35.5	13.7	50.8

er amino acids. Mutants W128L and W128C catalyze the formation of the two possible diastereomers (2*S*,3*S*)-**6** and (2*R*,3*S*)-**6** in comparable amounts. With the even less bulky MeHNL mutants W128A and W128V, however, (*R*) selectivity dominates, preferably yielding the (2*R*,3*S*) diastereomer (Table 2).

MeHNL-catalyzed HCN addition to *rac*-2-methyl-3-phenyl-propion-aldehyde (*rac*-**7**) and *rac*-3-phenylbutyraldehyde (*rac*-**9**)

In the addition reactions described so far the bulky phenyl substituent in aldehydes **1** and **5** is very close to the reacting carbonyl group. We were therefore interested to investigate the influence of phenyl substituents in the 3-position of aldehydes on the stereochemistry of the MeHNL-catalyzed cyanohydrin formation.

The 3-phenyl-substituted aldehydes **7** and **9** were treated with HCN under standard conditions[9] with wtMeHNL and MeHNL mutants, respectively, to give the corresponding cyanohydrins **8** and **10** (Scheme 6).



Scheme 6. *MeHNL catalyzed HCN addition to 3-phenyl substituted aldehydes* rac-***7*** *and* rac-***9***.

As can be seen from Table 3, a phenyl substituent in the 3-position of the aldehyde has practically no influence on the stereoselectivity of the various MeHNL mutants. Both wtMeHNL and all investigated MeHNL mutants are (*S*)-selective. The formation of the (2*S*,3*R*)-**8** and (2*S*,4*R*)-**10** diastereomers occurs with high diastereoselectivity.

Conclusion

The active site and the channel entrance to the active site of wtMeHNL and MeHNL W128 mutants are well known from X-ray crystal-structure investigations.[1] By using this structural data, it is possible to rationalize the expected as well as the unexpected results of stereoselectivity of MeHNL-catalyzed HCN addition to 2-substituted aldehydes that contain a stereocenter adjacent to the carbonyl group. This will be discussed and rationalized by using the MeHNL-catalyzed reaction of *rac*-**1** as an example.

Table 3. *Stereoselectivity of HCN addition to* rac-***7*** *and* rac-***9*** *catalyzed by MeHNL W128 mutants, which give the diastereomeric cyanohydrins* ***8*** *and* ***10****, respectively.*

MeHNL mutants	Aldehyde *rac*-**7** Cyanohydrin **8** (2*S*,3*R*) *de* (%)	(2*S*,3*S*) *de* (%)	Aldehyde *rac*-**9** Cyanohydrin **10** (2*S*,4*S*) *de* (%)	(2*S*,4*R*) *de* (%)
wt	97	75	80	98
W128Y	97	84	96	>99
W128L	95	90	80	90
W128C	95	82	87	98
W128A	97	84	93	>99

The decisive transition state for the formation and cleavage of cyanohydrins is structurally more cyanohydrin-like with variations in the length of the hydrogen bonds formed and split during the course of the reaction.[1] Therefore, to explain the stereochemical results described so far, molecular modeling based on the defined X-ray structures of wtMeHNL (Figure 3) and the MeHNL W128A (Figure 4) mutant with the corresponding cyanohydrins as substrates was carried out.

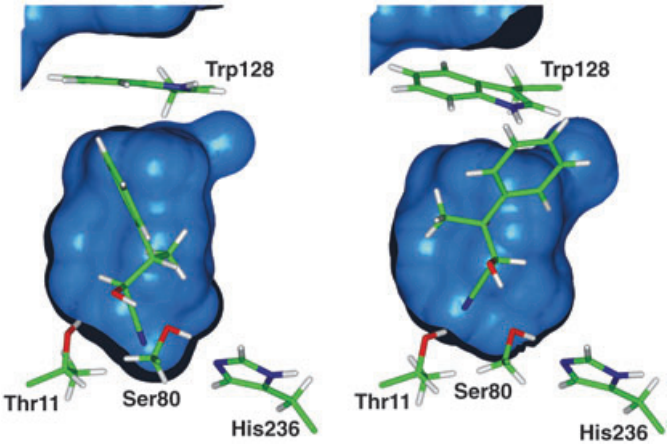


Figure 3. *Molecular modeling representation of the (2S,3S) and (2R,3S) diastereomers of cyanohydrin* ***2*** *in the active site of wtMeHNL.*

Since the (*R*) enantiomer of *rac*-**1** reacts (*S*)-selectively with the wild-type enzyme and all mutants investigated, it shall not be discussed further. Molecular modeling should, however, explain the inversion of stereoselectivity in the reaction of the (*S*) enantiomer of *rac*-**1** catalyzed by the W128A mutant. In Figure 3, the two possible cyanohydrin diastereoisomers of (*S*)-**1**, complexed in the active center of wtMeHNL, are visualized. Steric demands of the phenyl and methyl substituents are the decisive factors for the orientation of the cyanohydrin in the active site. The catalytic activity of the enzyme depends on the

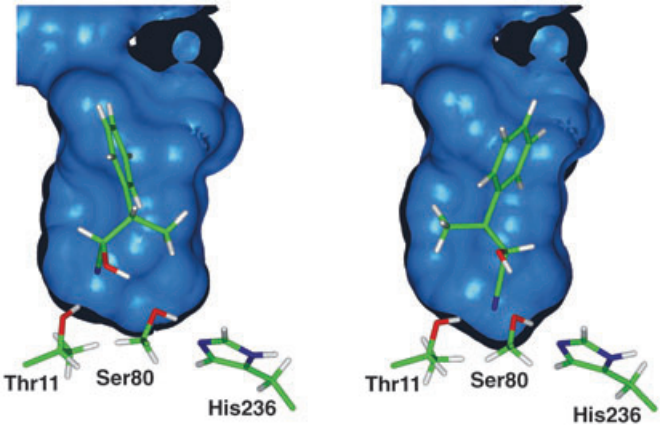


Figure 4. *Molecular modeling representation of the (2S,3S) and (2R,3S) diastereomers of cyanohydrin* ***2*** *in the active site of MeHNL W128A.*

strength of the hydrogen bridges between Ser80, Thr11, His236, and the cyanohydrin.[1c] For the (2*S*,3*S*) configuration, energetically favored hydrogen bonds are possible from Ser80 to His236, from the cyanohydrin to Ser80, and from Thr11 to the cyanohydrin (Figure 3). For the (2*R*,3*S*) configuration, however, there are no hydrogen bonds to Thr11, and the cyano group points away from the catalytic residues. Therefore the formation of the (2*R*,3*S*) diastereomer is disfavored. Since the channel entrance is blocked by Trp128, the diffusion of the aldehyde into the active site is relatively slow and rate-determining;[3] this causes the preferred formation of the (2*S*,3*S*) diastereomer.

The situation changes, however, for the active site of the MeHNL W128A mutant (Figure 4). In the sterically optimized positions, the cyano group of the (2*S*,3*S*)-configured diastereomer points away from the catalytic residues. A hydrogen bridge of the cyanohydrin to Ser80, is therefore not possible for the (2*S*,3*S*) diastereomer, whereas the (2*R*,3*S*) diastereomer is positioned in the correct orientation. In comparison to the (2*R*,3*S*) configuration, the specific activity of MeHNL W128A is approximately 450 times higher than the specific activity of wtMeHNL,[1d] therefore the conversion rate is determined by the favored (2*R*,3*S*) configuration of the transition state in the enzyme complex.

In summary, it is possible to explain and rationalize the inversion of (*S*) stereoselectivity of wtMeHNL to (*R*) selectivity of the MeHNL W128A mutant from crystal-structure-based molecular modeling. It is remarkable that site-directed mutagenesis was not performed in the active site of the enzyme but at the periphery.

Experimental Section

Material and methods: Melting points were determined on a Büchi SMP-20 and are uncorrected. Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 F (250 MHz) or ARX 500 (500 MHz) spectrometer in $CDCl_3$ with TMS as internal standard. Optical rotations were measured with a Perkin–Elmer polarimeter 241 LC in a thermostated glass cuvette ($l = 10$ cm). Chromatography was performed by using silica gel, grain size 0.040–0.063 mm (Fluka). Diastereomeric excess: GC separations were conducted by using 1) capillary glass columns (20 m) with OV 1701, carrier gas 0.4–0.6 bar hydrogen; 2) a Chiraldex B-PM (permethylated) column (30 m × 0.32 mm), carrier gas 0.6–1.0 bar hydrogen; or 3) a Chiraldex B-TA and G-TA column (30 m × 0.32 mm), carrier gas hydrogen. Aldehydes **1** and **9** are commercially available. Yields were not optimized.

***rac*-2-Phenylbutyraldehyde (*rac*-5)**: 1) A solution of *rac*-2-phenylbutyric acid (29.0 g, 177 mmol) in absolute Et_2O (400 mL) was added dropwise to a vigorously stirred suspension of $LiAlH_4$ (6.7 g, 177 mmol) in absolute Et_2O (100 mL) at a rate that ensured that the solvent continued to boil. After the reaction mixture had been stirred overnight at room temperature, H_2SO_4 (5%) was added to the ice-cooled mixture to resolve the precipitated aluminum salts. The organic layer was separated, dried (Na_2SO_4), and concentrated. Distillation under vacuum yielded 23.7 g *rac*-2-phenylbutanol (85%), b.p. 118 °C/23 mbar. ^{1}H and ^{13}C NMR spectra were consistent with the literature;[11] 2) A solution of *rac*-2-phenylbutanol (43 g, 200 mmol) in dichloromethane (400 mL) was added to an ice-cooled vigorously stirring suspension of pyridiniumchlorochromate (22.5 g, 200 mmol) in dichloromethane (100 mL). When the reaction was complete (GC control), the mixture was filtered over silica. The solvent was removed, and the crude product was distilled under vacuum to yield 17.3 g (78%) *rac*-**5**. b.p. 105 °C/28 mbar. ^{1}H and ^{13}C NMR spectra were consistent with the literature.[12]

***rac*-2-Methyl-3-phenylpropionaldehyde (*rac*-7)**: 1) 2-Methyl-3-phenylpropanol was prepared from *rac*-2-methylhydrocinnamic acid (15.0 g, 91.4 mmol) as described above for *rac*-2-phenylbutanol in 97% yield. b.p.: 127 °C/25 mbar. ^{1}H and ^{13}C NMR spectra were consistent with the literature.[13] 2) *rac*-**7** was prepared from *rac*-2-Methyl-3-phenylpropanol (13.3 g, 88.5 mmol) as described above for *rac*-**5** in 68% yield, b.p.: 100 °C/18 mbar. ^{1}H NMR and ^{13}C NMR-spectra were consistent with the literature[14]

Racemic cyanohydrins were prepared according to a known literature procedure.[15]

***rac*-2-Hydroxy-3-phenylbutyronitrile (*rac*-2)**: ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.46$ (d, $J = 7.15$ Hz, 1.5 H; CH_3), 1.47 (d, $J = 7.1$ Hz, 1.5 H; CH_3), 3.10–3.22 (m, 2 H; C3-H, OH), 4.48 (d, $J = 6.56$ Hz, 0.5 H; C2-H), 4.50 (d, $J = 6.17$ Hz, 0.5 H; C2-H), 7.15–7.41 (m, 5 H; H_{Ph}); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 15.99$, 16.03 (CH_3), 43.99, 44.25 (C3), 66.45, 66.77 (C2), 118.77, 118.87 (CN), 127.86, 127.91, 128.05, 128.19, 128.92, 139.06, 139.29 (C_{Ph}).[16]

***rac*-2-Acetoxy-3-phenylbutyronitrile (*rac*-2′)**: Acetic anhydride (45 mmol) was added to a solution of the crude aldehyde *rac*-**2** (30 mmol) in pyridine (45 mL). The reaction mixture was allowed to stand at 60 °C for 1 h. Then the reaction mixture was diluted with ethyl acetate (100 mL) and washed with diluted HCl until neutral and then with a saturated solution of $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), and concentrated. The crude product was distilled under vacuum to yield 4.7 g (77%) *rac*-**2′** (*rac-syn*/*rac-anti* = 60:40) as a colorless oil. b.p. 107 °C/0.01 mm; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.47$ (d, $J = 7.1$ Hz, 1.5 H; C4-H_3), 1.53 (d, $J = 7.07$ Hz, 1.5 H; C4-H_3), 2.06 (s, 1.5 H; $COCH_3$), 2.12 (s, 1.5 H; $COCH_3$), 3.23–3.35 (m, 1 H; C3-H), 5.43 (t, $J = 6.7$ Hz, 1 H; C2-H), 7.26–7.41 (m, 5 H; H_{Ph}).[17] ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 15.82$, 16.31 (CH_3), 20.28, 20.34 ($COCH_3$), 41.86, 41.95 (C3), 65.63, 66.05 (C2), 115.89, 116.00 (CN), 127.72, 127.91, 127.95, 128.05, 128.83, 128.92, 138.73, 138.86 (C_{Ph}), 168.89, 169.09 (COO).[17]

***rac*-2-Hydroxy-3-phenylpentanenitrile (*rac*-6)**: ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.80$–0.88 (m, 3H; C5-H_3), 1.73–2.10 (m, 2H; C4-H_2), 2.81–2.91 (m, 2H; C3-H, OH), 4.49–4.57 (m, 1H; C3-H), 7.22–7.41 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): 11.68, 11.74 (C5), 23.43, 23.65 (C4), 51.66, 51.99 (C3), 65.55 (C2), 118.88, 119.18 (CN), 127.84, 128.09, 128.61, 128.81, 128.9, 128.95, 137.41, 137.59 (C_{Ph}).

***rac*-2-Acetoxy-3-phenylpentanenitrile (*rac*-6′)**: Reaction conditions as for *rac*-**2**′. Yield: 5.4 g (83%) *rac*-**6**′ as a colorless oil (*rac-syn*/*rac-anti* = 65:35). b.p. 126 °C/0.01 mm; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 0.83–0.87 (m, 3H; C5-H_3), 1.77–1.98 (m, 2H; C4-H_2), 2.02 (s, 1.5H; $COCH_3$), 2.11 (s, 1.5H; $COCH_3$), 2.96–3.03 (m, 1H; C3-H), 5.47 (d, $J =$ 7.07 Hz, 0.5H; C2-H), 5.48 (d, $J = 6.57$ Hz, 0.5H,C2-H), 7.24–7.38 (m, 5H; H_{Ph}); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 11.57$, 11.61 (C5), 20.24, 20.37 ($OCOCH_3$), 23.37, 23.74 (C4), 49.32, 49.53 (C3), 65.03 (C2), 116.02, 116.21 (CN), 127.90, 128.05, 128.39, 128.53, 128.78, 128.83, 137.06, 137.19 (C_{Ph}), 168.89, 169.13 (COO); elemental analysis calcd (%) for $C_{13}H_{15}NO_2$ (217.27): C 71.87, H 6.96, N 6.45; found: C 71.71, H 7.01, N 6.24.

***rac*-2-Hydroxy-3-methyl-4-phenylbutyronitrile (*rac*-8)**: ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.07$ (d, $J = 6.87$ Hz, 1.5H; C4-H_3), 1.08 (d, $J =$ 6.72 Hz, 1.5H; C4-H_3), 2.08–2.28 (m, 1H; C3-H), 2.50–2.96 (m, 2H; $PhCH_2$), 3.58 (br s, 1H; OH), 4.29–4.36 (m, 1H; C2-H), 7.16–7.36 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 14.59$, 14.63 (CH_3), 37.92, 38.56, 39.74, 39.87 (C3, Ph*C*), 64.53, 65.32 (C2), 118.94, 119.51 (CN), 126.55, 126.58, 128.23, 128.62, 129.07, 129.14, 138.51, 138.95 (C_{Ph}).

***rac*-2-Acetoxy-3-methyl-4-phenylbutyronitrile (*rac*-8′)**: For reaction conditions see *rac*-**2**′. Yield: 5.8 g (89%) *rac*-**8**′ as a colorless oil (*rac-syn*/*rac-anti* = 47:53). b.p. 136 °C/0.1 mm; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.09$ (d, $J = 6.9$ Hz, 1.5H; C4-H_3), 1.12 (d, $J = 6.76$ Hz, 1.5H; C4-H_3), 2.11 (s, 1.5H; $OCOCH_3$), 2.14 (s, 1.5H; $OCOCH_3$), 2.20–2.39 (m, 1H; C3-H), 2.52–2.94 (m, 2H; $PhCH_2$), 5.17–5.21 (m, 1H; C2-H), 7.12–7.46 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 14.96$ (CH_3), 20.30, 20.35 ($OCOCH_3$), 37.61, 37.97, 38.09, 39.72 (C3, $PhCH_2$), 64.68, 65.22 (C2), 115.67, 116.21 (CN), 126.68, 126.80, 128.66, 128.74, 128.92, 129.02, 137.95, 138.33 (C_{Ph}), 169.07, 169.13 (COO); elemental analysis calcd (%) for $C_{13}H_{15}NO_2$ (217.27): C 71.87, H 6.96, N 6.45; found: C 71.76, H 7.01, N 6.27.

***rac*-2-Hydroxy-4-phenylpentanenitrile (*rac*-10)**: ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.30$ (d, $J = 7.0$ Hz, 1.5H; C5-H_3), 1.31 (d, $J = 6.96$ Hz, 1.5H; C5-H_3), 1.95–2.20 (m, 2H; C3-H_2), 2.92–3.11 (m, 1H; C4-H), 3.40 (br s, 1H; OH), 4.09–4.16 (m, 1H; C2-H), 7.17–7.35 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 22.18$, 22.26, 35.52, 36.04, 43.18, 43.25 (C5, C4, C3), 59.2, 60.21 (C2), 119.94, 120.18 (CN), 126.76, 126.88, 126.89, 127.09, 128.88, 144.36, 144.64 (C_{Ph}).

***rac*-2-Acetoxy-4-phenylpentanenitrile (*rac*-10′)**: For reaction conditions see *rac*-**2**′. Yield: 4.6 g (71%) *rac*-**10**′ as a colorless oil (*rac-syn*/*rac-anti* = 50:50). b.p. 123 °C/0.1 mm; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.33$ (d, $J = 6.96$ Hz, 3H; C5-H_3), 2.01 (s, 3H; $OCOCH_3$), 2.06 (s, 3H; $OCOCH_3$), 2.16–2.26 (m, 2H; C3-H_2), 2.87–3.04 (m, 1H; C4-H), 5.00–5.03 (m, 1H; C2-H), 7.12–7.36 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): 20.29, 20.33, 22.14, 22.62, 35.98, 36.44, 40.12, 40.46, (C5, C4, C3, $OCOCH_3$), 59.64, 60.46 (C2), 116.89, 117.06 (CN), 126.82, 126.91, 127.11, 127.14, 129.00, 129.04, 144.07, 144.09 (C_{Ph}), 169.07, 169.10 (COO); elemental analysis calcd (%) for $C_{13}H_{15}NO_2$ (217.27): C 71.87, H 6.96, N 6.45; found: C 71.58, H 7.01, N 6.57.

Enzyme-catalyzed conversion of aldehydes to cyanohydrins and determination of enantiomeric excess and conversion percentages were carried out as described in the literature.[3]

(2*S*,3*RS*)-2-Hydroxy-3-phenylbutyronitrile [(2*S*,3*RS*)-2]: *syn*-(2*S*,3*R*)/*anti*-(2*S*,3*S*) = 54:46, $[\alpha]_D^{20} = -12.8°$ ($c = 1.0$, $CHCl_3$); ^{1}H and ^{13}C NMR spectra were consistent with *rac*-**2**.

(2*S*,3*SR*)-2-Hydroxy-3-phenylpentanenitrile [(2*S*,3*SR*)-6]: *syn*-(2*S*,3*R*)/*anti*-(2*S*,3*S*) = 53:47, $[\alpha]_D^{20} = -18.9°$ ($c = 1.0$, $CHCl_3$); ^{1}H and ^{13}C NMR spectra were consistent with *rac*-**6**.

(2*S*,3*SR*)-2-Hydroxy-3-methyl-4-phenylbutyronitrile [(2*S*,4*SR*)-8]: *syn*-(2*S*,3*R*)/*anti*-(2*S*,3*S*) = 53:47, $[\alpha]_D^{20} = +18.6°$ ($c = 1.0$, $CHCl_3$). ^{1}H NMR and ^{13}C NMR-spectra were consistent with *rac*-**8**.

(2*S*,4*SR*)-2-Hydroxy-4-phenylpentanenitrile [(2*S*,3*SR*)-10]: *syn*-(2*S*,4*R*)/*anti*-(2*S*,4*S*) = 55:45, $[\alpha]_D^{20} = +17.7°$ ($c = 1.0$, $CHCl_3$); ^{1}H and ^{13}C NMR spectra were consistent with *rac*-**10**.

(2*S*,3*RS*)-2-*p*-bromobenzoyloxy-3-phenylbutyronitrile [(2*S*,3*RS*)-3]: *p*-bromobenzoyl chloride and pyridine were added to a solution of (2*S*,3*RS*)-**2** (4.00 g, 24.8 mmol) in dichloromethane (50 mL) and stirred for 2 h. When the reaction was completed (GC control), the reaction mixture was diluted with Et_2O (150 mL) and washed with saturated solutions of $NaHCO_3$ and NaCl. The organic layer was separated, dried (Na_2SO_4), and concentrated. The crude product consisted of 2 diastereomers in 46:54 ratio (GC), which were separated by column chromatography on silica gel. The eluent was changed slowly from pure petroleum ether to a mixture of petroleum ether and ethyl acetate (98:2).

Fraction 1: yield (*anti*-(2*S*,3*S*)-**3**) 2.57 g (31%); m.p. 79–80 °C; $[\alpha]_D^{20} = -60.6°$ ($c = 2.4$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.60$ (d, $J =$ 7.1 Hz, 3H; CH_3), 3.39–3.50 (m, 1H; C3-H), 5.65 (d, $J = 6.7$ Hz, 1H; C2-H), 7.26–7.38 (m, 5H; H_{Ph}) 7.56–7.83 (m, 4H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 16.0$ (CH_3), 42.2 (C3), 66.9 (C2), 115.9 (CN), 127.2, 127.8, 128.2, 129.0, 129.4, 131.4, 132.1, 138.7 (C_{Ph}), 163.6 (COO).

Fraction 2: yield (*syn*-(2*S*,3*R*)-**3**) 2.57 g (31%); m.p. 68–70 °C; $[\alpha]_D^{20} = -34.7°$; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.56$ (d, $J = 7.1$ Hz, 3H; CH_3), 3.38–3.49 (m, 1H; C3-H), 5.67 (d, $J = 6.7$ Hz, 1H; C2-H), 7.28–7.41 (m, 5H; H_{Ph}), 7.58–7.86 (m, 4H; H_{Ph}); ^{13}C NMR (63 MHz, $CDCl_3$): 16.3 (CH_3), 42.0 (C3), 66.3 (C2), 115.8 (CN), 128.0, 128.2, 128.9, 129.4, 131.4, 132.1, 138.7 (C_{Ph}), 163.6 (COO).

Single crystals for X-ray analysis could be obtained from toluene (*anti*-(2*S*,3*S*)-**3**) and diisopropyl ether (*syn*-(2*S*,3*R*)-**3**).[10] Elemental analysis calcd (%) for $C_{17}H_{14}NO_2Br$ (344.21): C 59.32, H 4.10, N 4.07, Br 23.21; found: C 59.28, H 4.13, N 4.19, Br 23.06.

***syn*-2-Hydroxy-3-phenylbutyric acid (*syn*-4)**: 4.5 g (28 mmol) of crude **2** from the MeHNL W128A-catalyzed conversion of *rac*-**1** was heated to 60 °C with concentrated HCl (30 mL) for 12 h. The reaction mixture was diluted with H_2O (30 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were extracted with saturated $NaHCO_3$ (3 × 50 mL). The combined aqueous layers were acidified with concentrated HCl (pH 1) and again extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Traces of solvent were removed under vacuum to yield **4** (4.35 g, 87%) as a colorless oil that began to crystallize after time.[10] m.p. 89 °C (from $CHCl_3$), $[\alpha]_D^{20} = 0°$ ($c = 2.6$, $CHCl_3$), ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.19$ (d, $J = 7.1$ Hz, 3H; CH_3), 3.05–3.19 (m, 1H; C3-H), 3.38 (br s, 1H; OH), 4.03 (d, $J = 5.0$ Hz, 1H; C2-H), 7.14–7.36 (m, 5H; H_{Ph}); ^{13}C NMR (63 MHz, [D_6]DMSO) 15.3 (CH_3), 42.3 (C3), 74.5 (C2), 126.1, 127.7, 127.9, 143.7 (C_{Ph}), 174.7 (COO); elemental analysis calcd (%) for $C_{10}H_{12}O_3$ (180.20): C 66.65, H 6.71; found: C 66.70, H 6.71.

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[10] CCDC 246200–246202 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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